NON LINEARITY IN DOMINANT LETHALS INDUCED WITH IRRADIATION IN DROSOPHILA MELANOGASTER

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It has long been known that linearity between the frequency of lethal mutations induced by X-rays is an established fact in dose frequency curves. The pioneering investigations of Oliver (1930) cast some doubts about the mutational mechanism involved in the structural breakage origin of some lethals, because his results showed perfect linearity over the whole range of doses including the highest. Data has been produced which demonstrated that chromosomes changes increase more rapidly than linearity consents (Timofeeff-Ressovsky, 1930; Lea and Catcheside, 1945; Herskowitz, 1946; Muller, Altenburg, Meyer, Edmondson and Altenburg, 1954).

Since 1952 Luning has shown the importance of differential radiosensitivity at the time of irradiation as a factor in the production of chromosome abnormalities which yield lethals. Previous irradiation studies on mature spermatozoa of *Drosophila melanogaster*, used sperm samples from mass matings or from large mating periods (4-7 days) and at any rate no particular care was taken of the age of the irradiated samples or to their homogeneity. It is, therefore, quite desirable to reinvestigate the lethal frequency dosage ralationship for chromosome abnormalities.

The present investigation takes Luning's warning into consideration to study by way of the daily brood method the successive stages of gametogenesis and their susceptibility to produce dominant lethals at various doses.

MATERIALS AND METHODS

Three days old adult *D. melanogaster* were irradiated with 150r, 300r, 600r, and 1200r. An X-ray therapy Ghilardoni machine delivering at 200r per minute at 220 kv and 12 mA with an inherent filtration of 4mm Al was used. Dosimetry was done with a Siemens chamber. *D. melanogaster* Oregon R males after been irradiated are placed singly with 3 females of their own strain in a vial containing a spoon with the usual corn, sugar and agar medium plus a little charcoal to darken the food and facilitate the egg counts. The females are left to copulate with the irradiated males for 24 hrs. after which the spoon is changed and the eggs counted passed to normal food vials. Every day a new batch of virgin females are crossed to the treated males. In this way daily samples of sperms can be analyzed for the occurence of dominant lethals.

RESULTS

Figure 1 and table 4 present the results of dominant lethals induced in mature spermatozoa. These are the samples from the first 3 days after irradiation. When the males were irradiated the sperms cells first used in fertilization were mature at the time of the treatment. Table 2 shows the effect of sperm age on the frequencies of dominant lethals induced. In order to distinguish whether the effect of age resides on the time from fertilization to irradiation or whether it is the time from irradiation to insemination table 1 and table 3 are presented. They suggest how the different sperm batches have an influence on the percentage of dominant lethals induced.

Furthermore, figure 1 indicates the dose frequency curve from the various doses and for mature sperms cells of the first 3 days of copulations. Figure 2 presents the entire spectrum of radiosensitivity at two critical doses (600r and 1200r).

DISCUSSION

The results show that there is a difference between the sperm batches Ib and IIa, IIa and IIb, and IIb and IIIa having similar age from irradiation to fertilization; this finding, as discussed by Baker and von Halle (1953) does not support the differential breakage hypothesis. Furthermore, there is a clear difference between dominant lethals induced in the first day sample and those induced in later days. The results also show a considerable departure from the first power of the dose at high doses. In the present experiments although mature sperms are used the nature of the 3 days samples are discussed below.

A word should be said about the experiments made by Harris (1929), Timofeeff-Ressovsky (1931) and Demerec and Kaufmann (1941) about the time of sperm exhaustion and the variations in the rates of dominant lethals. The drop in the percentage of sex-linked lethals which these authors registered between the 15 and the 16th. day after the X-ray treatment could not have been very precise due to the large mating periods. That is, since the male was kept with the female for several days, the exhaustion of the mature sperm supply is in relation to the number of copulations which that male could accomplish in that period of time To resolve the sperm samples sensitivity properly daily sperms batches are needed to follow the sensitivity spectrum (see table 5). Thus Timofeeff-Ressovsky's five day mating period included eggs which were fertilized in the first copulations by spermatozoa still of the mature zone, while those eggs layed from later copulations included spermatids and probably even immature spermatogonia germ cells. The drop could have been at the 7th. day as indicated by our results, but with their five day mating periods this peak could not be realized. To this effect Luning (1952) rightly points out a discrepancy in the conclusions from the results of table 1 of Demerec and Kaufmann (1941). A lower rate of hatch is also found by these authors at the 7th. day if the results of daily copulations are put together.

Several investigators (Catcheside, 1938, and Eberhardt, 1939) have found that gross structural changes varied as a power of dose of one. Nevertheless, others (Timofeeff-Ressovsky, 1939, and Muller, 1940) have confirmed the $^{3}/_{2}$ power of the dose for doses ordinarily used. It is a well established fact that single breakage varies in a simple linear manner with the dose; this is evident when X chromosome losses derived from irradiated X and which result from dicentric and acentric isochromosomes. The natural conclusion is that single breaks come from single ionizations or activations. The induction of dominant lethals due to bridge formation at the zygote follows linearity (Muller and Pontecorvo, 1941). This effect occurring at lower doses of X-rays could be responsible for our own results at 150, 300 and 600r.; but not so for the doses from 600 to 1200r. The effect close to the $^{3}/_{2}$ power of the dose is found in spermatid sensitivity (see figure 2). Thus, at higher doses, multiple breakage causing aneucentric structural changes increase at a power greater than one. Therefore, dominant lethals at the most sensitive stage of spermatogenesis probably consist of more than single breaks.

Muller, Herkowitz, Abrahamson and Oster (1954) suggest germinal selection for sperm cells which carry most lethals. The same criterion may be applied to dominant lethals: the sperms available for fertilization will be the ones least affected by the irradiation. Therefore, for a sample to show differential response with respect to the dose it must be heterogeneous: it must have more resistant and less resistant germ cells.

However, at lower doses it is possible to get a 1:1 relationship in the dose-frequency curves. At these doses there are only single breaks and the selective effects of different sensitivities in heterogeneous samples are too weak and the curve in this section will appear linear.

SUMMARY

The design in the experiments permitted the study of daily batches of sperm cells. Counts of dominant lethals induced with X-irradiation consented a more detail analysis of the dose-frequency hypothesis for chromosome abnormalities.

RESUMEN

El nuevo diseño experimental aquí presentado hizo posible el estudio de cada uno de los grupos de células germinales en la espermatogénesis de *Drosophila melanogaster* Oregon R. Los recuentos de los letales dominantes inducidos con irradiación X permitieron el análisis detallado de la hipótesis sobre dosis-frecuencia para anormalidades cromosómicas.

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TABLE 1. DESIGN OF EXPERIMENTS

	Irradiation to Insemination	Irradiation to Fertilization
Sperm batch 1a	0-24 hrs.	24-48 hrs.
Sperm batch 1b	0-24 "	48-72 "
Sperm batch 11a	24-48 "	48-72 "
Sperm batch 11b	24-48 "	72-96 "
Sperm batch 111a	48-72 "	72-96 "
Sperm batch 111b	48-72 "	96-120 "

TABLE 2. EFFECT OF SPERM AGE ON THE FREQUENCY OF DOMINANT LETHALS INDUCED

Age in Days

	1		2		3	
Dose	Eggs counted	% hatch	Eggs counted	% hatch	Eggs counted	% hatch
150r	683	80.0	458	86.0	349	84.2
300r	477	73.2	549	72.7	470	72.1
600r	446	50.6	349	64.2	133	65.4
1200r	612	32.2	343	34.9	396	45.9

TABLE 3. PERCENTAGE OF DOMINANT LETHALS INDUCED IN DIFFERENT SPERM BATCHES

% hatch in different sperm batches

Dose	la	lb	lla	llb	llla	lllb
150r	51.6	87.8	73.8	88.2	86.7	80.0
300r	61.3	78.1	69.5	75.1	87.0	62.9
600r	61.5	46.4	65.9	65.4	52.3	67.3
1200r	28.9	32.6	29.1	31.7	52.4	41.9

TABLE 4. PERCENTAGE OF DOMINANT LETHALS AT VARIOUS DOSES

In this table the total hatch from the eggs fertilized by sperm samples from the first 3 days after irradiation are added. The control % hatch was 90.94.

	Doses			
	150r	300r	600r	1200r
Eggs	1490	1496	928	1351
Hatch	1233	1086	537	499
% lethals	8.19 + 0.93	18.35 + 0.99	33.94 + 0.16	53.01 + 0.13

TABLE 5. DAILY PERCENT SURVIVAL AT 600r AND 1200r.

day	dose	eggs	% hatch
	600r	441	54.0
1	1200r	612	30.8
	600r	349	65.7
2	1200r	343	30.4
	600r	133	59.8
3	1200r	396	47.2
	600r	351	59.5
4	1200r	812	61.4
	600r	470	76.8
5	1200r	1179	64.6
	600r	930	73.6
6	1200r	1161	50.1
	600r	300	52.0
7	1200r	268	21.7
	600r	1256	55.2
8	1200r	1278	18.5
	600r	1638	49.6
9	1200r	1864	15.0

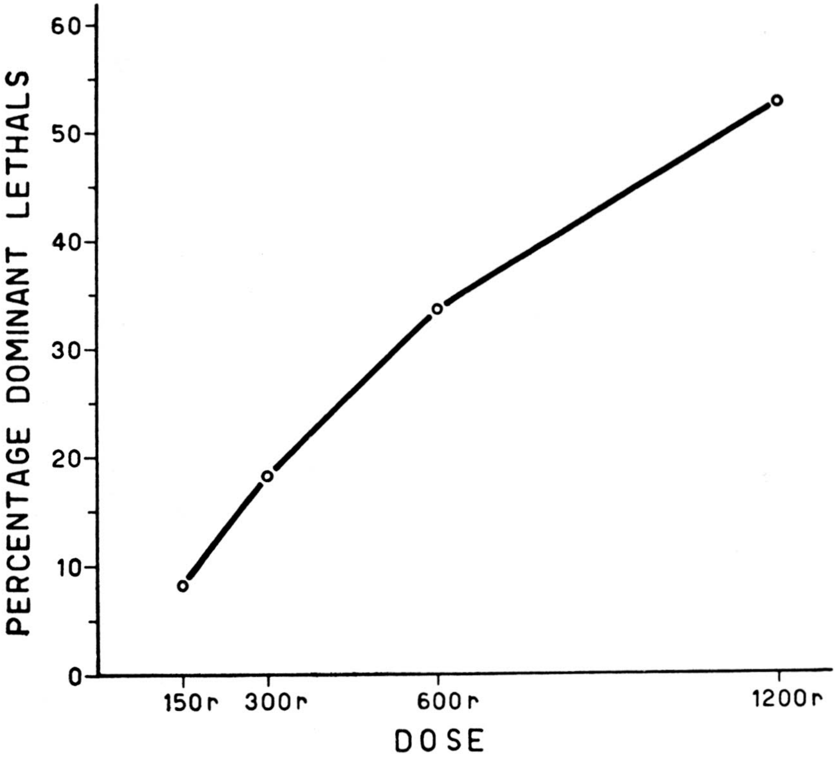

Fig. 1- Dose versus dominant lethals in *Drosophila melanogaster*.-

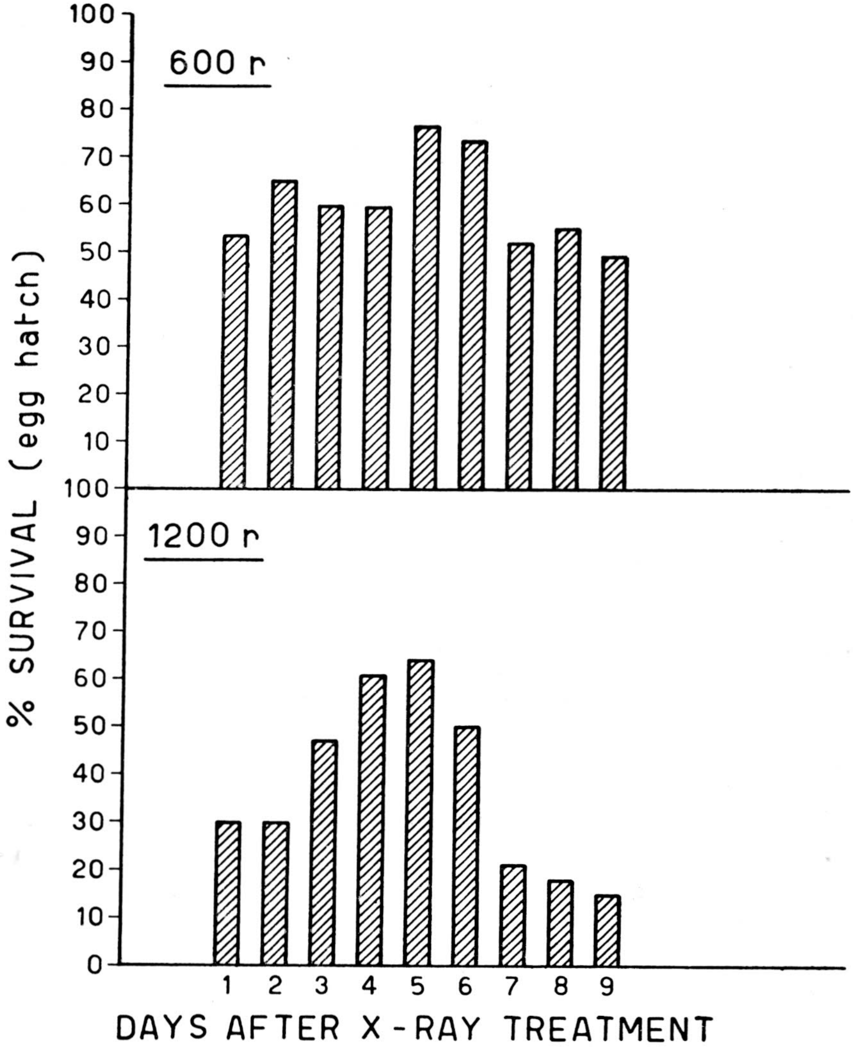

Fig. 2 – Testis sensitivity to X-ray doses (600r and 1200r) counted as dominant lethals induced.–